Nalidixic Acid Resistance Influences Sensitivity to Ionizing Radiation among *Salmonella* Isolates†

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ABSTRACT

Nalidixic acid (Nal) resistance has been used as a selective marker for studies of pathogen-inoculated fruits and vegetables. A collection of 24 *Salmonella* isolates were screened for natural resistance to Nal (50 μg/ml). The resistance to ionizing radiation was determined and compared for i) three naturally Nal-resistant (Nal^R) strains, ii) three naturally Nal-sensitive (Nal^S) strains, and iii) three strains derived from Nal^S strains that were made resistant to Nal (Nal^{Ri}) by successive culturing and selection in Nal-amended broth. The radiation D_{10}-values (the radiation dose required to achieve a 1-log reduction in population) were determined in buffer solution and in orange juice. D_{10}-values were significantly ($P < 0.05$) different among the *Salmonella* isolates tested. When considered as a group, Nal^R isolates were significantly more sensitive to ionizing radiation than Nal^S isolates in both media tested. In buffer, D_{10} of Nal^R was 0.210 kGy versus 0.257 kGy for Nal^S. In orange juice, D_{10} of Nal^R was 0.581 versus 0.764 for Nal^S. Inducing resistance to Nal altered the response to irradiation. D_{10}-value of Nal^{Ri} was 0.234 kGy in buffer, a 9% reduction relative to Nal^S parents. In orange juice, the D_{10}-value of Nal^{Ri} was 0.637 kGy, a reduction of 17% relative to Nal^S parents. These results suggest that natural and/or induced resistance to Nal may predispose *Salmonella* isolates to greater sensitivity to ionizing radiation, and that this effect is influenced by the suspending medium and by the nature of the isolates evaluated.

Nonpasteurized orange juice has been a recurrent vector for *Salmonella.* The significance of this food safety problem was recently underscored by a multistate outbreak of *Salmonella* Typhimurium in 2005 *(13, 20).* Human pathogens such as *Salmonella* serovars, *Listeria monocytogenes,* and *Escherichia coli* O157:H7 are increasingly associated with fresh and fresh-cut fruits and vegetables *(7, 8, 18).* The relatively high level of background microflora on fresh produce can complicate evaluation of antimicrobial interventions applied to these products *(15).* Selective media are often used to distinguish surviving pathogens from nonpathogenic background microflora, but can suppress the growth of bacteria which have sustained sublethal injury *(17).* The use of pathogenic strains which became antibiotic resistant is one common approach in inoculation studies *(3, 4).* This approach must be validated by demonstrating that the response of the antibiotic resistant daughter strains to the intervention being tested is similar to that of the original antibiotic-sensitive parent strain. Resistance to the quinolone antibiotic, nalidixic acid (Nal), was validated for use as a marker in studies of chemical interventions and growth parameter measurements *(3, 19).* In contrast, several Nal-resistant isolates of *E. coli* O157:H7 were shown to be significantly more sensitive to ionizing radiation that the Nal-sensitive parent strains from which they were derived *(14).* The use of this marker in studies of irradiated *E. coli* O157:H7 has the potential to lead to overestimates of the efficacy of irradiation in laboratory- and pilot-scale studies. Low doses of ionizing radiation are known to effectively reduce the level of pathogenic bacteria on fresh products—this efficacy is influenced by temperature, type, and maturity of subtending vegetable examined and other factors *(4, 15).* Accurate determination of the radiation D_{10} is the basis for accurate and effective application of ionizing radiation in commercial processing.

This study was done to determine i) the extent of resistance to Nal in a collection of foodborne-illness–related isolates of *Salmonella,* ii) the radiation resistance of naturally Nal-sensitive (Nal^S) versus naturally Nal-resistant (Nal^R) *Salmonella* isolates, and iii) the radiation resistance of subisolates of Nal^S parent isolates which have been induced to be Nal resistant (Nal^{Ri}).

MATERIALS AND METHODS

Microorganisms. A collection of 24 *Salmonella* isolates were obtained from Dr. Ethan Solomon (DuPont Experimental Station, Wilmington, Del.) and maintained in tryptic soy broth (TSB; Difco, Detroit, Mich.) at 4°C until use. The source of the isolates is presented in Table 1. The isolate collection was screened for resistance to Nal (Sigma Chemical Company, St. Louis, Mo.) as follows. Fresh cultures were grown from stocks by inoculation of fresh sterile TSB and incubation for 18 h at 37°C. The resulting culture had a cell density of ~10^9 CFU/ml. TSB was amended with sterile filtered (0.22 μm) Nal in concentrations of 0 (control), 10, 20, 30, 40, or 50 μg/ml. Concentrations were chosen based on the method of Taormina and Beuchat, with

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TABLE 1. *Source and MICs of Nal for 24* Salmonella *isolates*

Salmonella isolate	Source	Nal MIC (μg/ml)	Status
Anatum F4317	Sprouts	10	Nal^S
Baildon 61-99	Tomato	>50	Nal^R
Bredney 3VIPHE	Alfalfa seed	10	Nal^S
Enteritidis 15159	Orange juice	>50	Nal^R
Gaminara 02-615	Cantaloupe	10[a]	Nal^S
Gaminara F2172	Orange juice	>50[a]	Nal^R
Hdalgo 02-517-2	Cantaloupe	>50	Nal^R
Mbandaka 00916-1	Cantaloupe	10	Nal^S
Mbandaka RV1DHE	Alfalfa seed	>50	Nal^R
Michigan	Cantaloupe	>50[a]	Nal^R
Montevideo G4639	Tomato	10	Nal^S
Muenchen HERV2C	Alfalfa	>50	Nal^R
Newport 02-216	Cantaloupe	>50	Nal^R
Poona 418	Octopus	>50	Nal^R
Poona PTVS-1	Cantaloupe	10[a]	Nal^S
Poona G91-1574	Cantaloupe	>50	Nal^R
Poona 953	Ovine meat	>50[a]	Nal^R
Poona 348	Cantaloupe	10	Nal^S
St. Paul 02-517-2	Cantaloupe	>50	Nal^R
St. Paul FSIS-039	Beef	>50	Nal^R
Stanley HO58	Sprouts	10	Nal^S
Typhimurium DT104	Meat	10[a]	Nal^S
Typhimurium RO45	Cantaloupe	10	Nal^S
Worthington TX31	Alfalfa seed	>50	Nal^R

[a] Selected for use in irradiation studies.

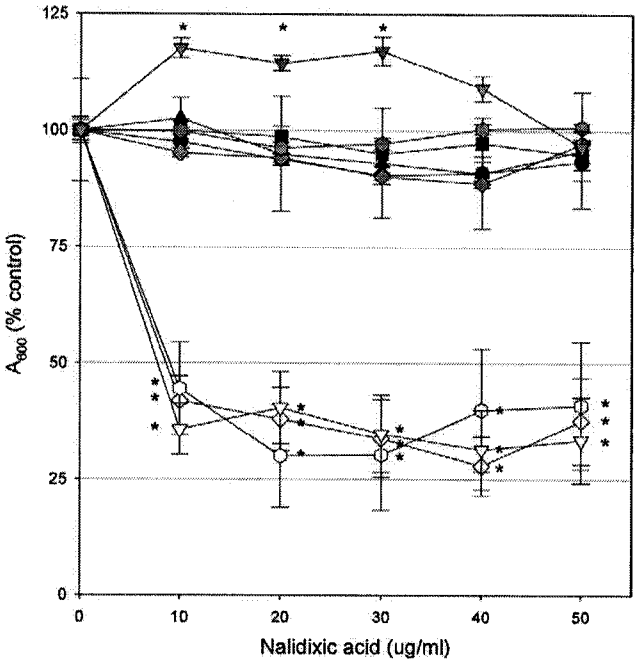

FIGURE 1. *Growth of* Salmonella *Gaminara F2172 (Nal^R, black circle),* Salmonella *Michigan (Nal^R, black square),* Salmonella *Poona 953 (Nal^R, black triangle),* Salmonella *Gaminara 02-615 (Nal^S, white diamond),* Salmonella *Poona PTVS-1 (Nal^S, white hexagon),* Salmonella *Typhimurium DT104 (Nal^S, white inverted triangle), and induced Nal^{Ri} isolates* Salmonella *Gaminara 02-615 (Nal^{Ri}, gray diamond),* Salmonella *Poona PTVS-1 (Nal^{Ri}, gray hexagon), and* Salmonella *Typhimurium DT104 (Nal^{Ri}, gray inverted triangle) in Nal-amended tryptic soy broth. Data points indicated by an asterisk (*) are significantly different ($P < 0.05$) from the respective control. Bars indicate SE ($n = 4$).*

the highest concentration of 50 μg/ml being above the clinical definition of resistance for Nal (i.e., 32 μg/ml) *(19).* Aliquots of amended TSB were dispensed into 96-well plates (200 μl per well) such that a given column contained one well of each concentration. In a given column, each well was inoculated with 10 μl of fresh culture of a single isolate. The plates were read for absorbance at 600 nm. The plates were then covered and incubated for 18 h at 37°C with gentle shaking, and read again for absorbance at 600 nm to establish turbidity, indicating growth of the culture at each Nal concentration. The evaluation was repeated in four separate trials, and the absorbance data pooled. Absorbance at each test concentration was compared to that of the control (0 μg/ml) using a one-way analysis of variance (ANOVA, Student-Neuman-Keuls test, SigmaStat, version 2.0, SPSS, Inc., Chicago, Ill.) to identify the MIC. An isolate was identified as resistant or sensitive based on the MIC.

Isolates whose growth was inhibited by the lowest concentration tested (10 μg/ml) were considered Nal^S. Isolates which grew at concentrations above 32 μg/ml (the clinical limit for resistance to Nal) were determined to be Nal^R (Table 1). Three Nal^R isolates (*Salmonella* Gaminara F2172, *Salmonella* Michigan, and *Salmonella* Poona 953) and three Nal^S isolates (*Salmonella* Gaminara 02-615, *Salmonella* Poona PTVS-1, and *Salmonella* Typhimurium DT104) were chosen for the subsequent radiation sensitivity experiments.

To compare the effect of induced Nal resistance from natural Nal resistance, the three Nal^S isolates were individually recultured in successively higher Nal concentrations to create Nal^R daughter strains, according to the method of Taormina and Beuchat *(19).* Solutions (5 ml) of sterile TSB were amended to 5, 10, 20, 30, 40, and 50 μg/ml using sterile Nal stock solutions. Every 24 h over a period of 6 days, 200 μl of culture was taken from the lower concentration solution and used to inoculate the next higher concentration solution. Cultures were grown at 37°C, with gentle shaking. To distinguish these induced resistant isolates from the naturally resistant isolates, they were designated Nal^{Ri}.

Once the test strains had been identified, the growth of the three Nal^R, Nal^S, and Nal^{Ri} isolates in TSB amended with 10, 20, 30, 40, or 50 μg of Nal was compared with the growth of their respective controls (0 μg/ml) using the 96-well plates as described above, with absorbance measured at 600 nm (Fig. 1). The growth confirmation was repeated in separate plates three times, and the data pooled.

D_{10} in solution. To determine the D_{10}-value in a neutral buffer solution, Nal^R, Nal^S, and Nal^{Ri} isolates were grown overnight (37°C) in 10-ml tubes of unamended TSB. The cultures were centrifuged (5,000 × *g*, 10 min) to recover the cells. The supernatant was discarded and the cells were resuspended in Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, Conn.), in 10-ml aliquots of ~10^8 CFU/ml. To more clearly delineate the effects of antibiotic resistance, the cultures were evaluated as individual isolates, rather than as a multi-isolate cocktail. The tubes were stored at 4°C until irradiation, typically 30 to 60 min.

D_{10} in orange juice. In a separate series of experiments to determine the D_{10}-value in orange juice, frozen concentrated orange juice containing pulp was reconstituted and inoculated according to Niemira *(13).* Briefly, the orange juice concentrate was aseptically reconstituted with 1,000 ml of distilled water per 355 ml of frozen concentrate, resulting in juice with a pH of 3.87.

Aliquots of 0.4 ml working culture per 10 ml juice preparation to be inoculated were centrifuged at 5,000 × *g* for 10 min to pelletize the cells. The TSB supernatant was discarded and the pelletized cells resuspended with part of a 10-ml aliquot of juice using a vortex mixer. The remainder of the 10-ml aliquot was added and the sample vortexed to fully suspend the cells. As with BPB, the cultures were evaluated as individual isolates. The samples were refrigerated (4°C) until irradiation, typically 30 to 60 min.

Irradiation. The inoculated solutions were treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8, or 1.0 kGy. The irradiation was conducted at 4°C. Temperature control was maintained during irradiation by injection of gas coming from liquid nitrogen into the sample chamber. The samples were irradiated using a Lockheed-Georgia cesium-137 self-contained gamma radiation source (Lockheed-Georgia, Marietta, Ga.), at a dose rate of 5.44 kGy/h. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology (Gaithersburg, Md.). Alanine pellets (Bruker, Inc., Billarica, Mass.) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. Actual dose was typically within 5% of the nominal dose.

Sampling. After irradiation, the solutions were immediately serially diluted with sterile BPB. After dilution, 1-ml samples were taken and pour plated with tryptic soy agar (TSA; Difco). Three plates per dilution were incubated at 37°C for 24 h and counted with a calibrated AccuCount 1000 automated colony counter (Biologics, Gainsville, Va.). The data for each sample were normalized against the control and plotted as the log reduction using the nominal doses. Each experiment was performed three times, and the data for each isolate pooled. The slopes of the individual survivor curves were calculated with linear regression (SigmaPlot 5.0, SPSS, Inc., Chicago, Ill.) and compared using analysis of covariance (ANCOVA; Excel 97, Microsoft Corp., Redmond, Wash.). The ionizing radiation D_{10}-value was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp., Ottawa, Ontario, Canada). For comparisons of the D_{10} of each group of isolates (Nal^R, Nal^S, and Nal^{Ri}), the D_{10}-values were pooled and compared using ANOVA.

RESULTS

Of the 24 *Salmonella* isolates examined, 10 were determined to be Nal^S based on growth inhibition by low concentrations of Nal (10 μg/ml). The remaining 14 isolates were not significantly inhibited by the highest concentration tested (50 μg/ml), and were determined to be Nal^R (Table 1). There was no discernible relationship between an isolate's source and its resistance to Nal. Both Nal^S and Nal^R groupings contained isolates from cantaloupe, alfalfa, tomato, and meats. Depending on the species, different isolates were seen to have Nal resistance that differed (e.g., *Salmonella* Poona, *Salmonella* Gaminara, *Salmonella* Mbandaka) or did not differ (e.g., *Salmonella* St. Paul, *Salmonella* Typhimurium).

The three Nal^R isolates selected for irradiation studies (*Salmonella* Gaminara F2172, *Salmonella* Michigan, and *Salmonella* Poona 953) grew at all concentrations tested (Fig. 1). The Nal^S isolates (*Salmonella* Gaminara 02-615, *Salmonella* Poona PTVS-1, and *Salmonella* Typhimurium DT104) were inhibited at all concentrations (Fig. 1). After induction of resistance, the three Nal^{Ri} isolates grew at all concentrations (Fig. 1). One Nal^{Ri} isolate, *Salmonella* Typhimurium DT104-Nal^{Ri}, had growth at 10, 20, and 30 μg/ml Nal that was slightly, though significantly, higher than that of the control. The resistance to Nal was retained in the Nal^{Ri} isolates after subsequent subculturing in the absence of Nal.

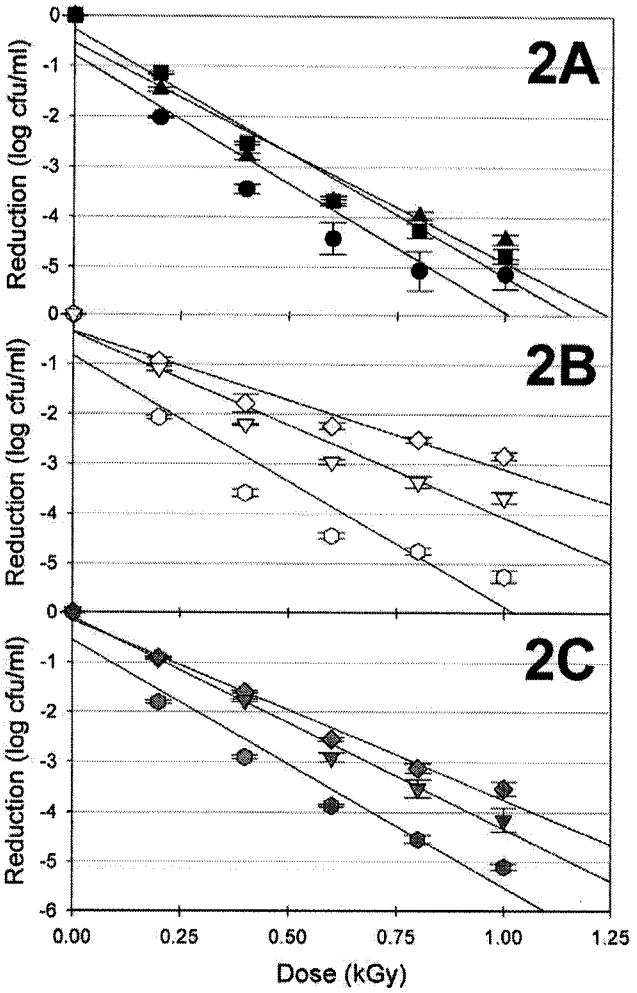

FIGURE 2. *Inactivation of* Salmonella *in phosphate buffer by irradiation. (A) Nal^R isolates* Salmonella *Gaminara F2172 (black circle),* Salmonella *Michigan (black square), and* Salmonella *Poona 953 (black triangle). (B) Nal^S isolates* Salmonella *Gaminara 02-615 (white diamond),* Salmonella *Poona PTVS-1 (white hexagon), and* Salmonella *Typhimurium DT104 (white inverted triangle). (C) Nal^{Ri} isolates* Salmonella *Gaminara 02-615 (gray diamond),* Salmonella *Poona PTVS-1 (gray hexagon),* Salmonella *Typhimurium DT104 (gray inverted triangle). Bars indicate SE* (n = *9 per data point).*

In buffer solutions, irradiation reduced the viable population of Nal^R (Fig. 2A), Nal^S (Fig. 2B), and Nal^{Ri} (Fig. 2C) isolates. The reduction was dose-dependent, with linear regression R^2 values ranging from 0.85 to 0.99. The resultant D_{10}-values were significantly different ($P < 0.05$) among the isolates. The Nal^R isolates had D_{10}-values ranging from 0.196 to 0.229 kGy, generally lower than the D_{10} of the Nal^S isolates, which ranged from 0.199 to 0.362 kGy (Fig. 3). When the data were pooled for each group of

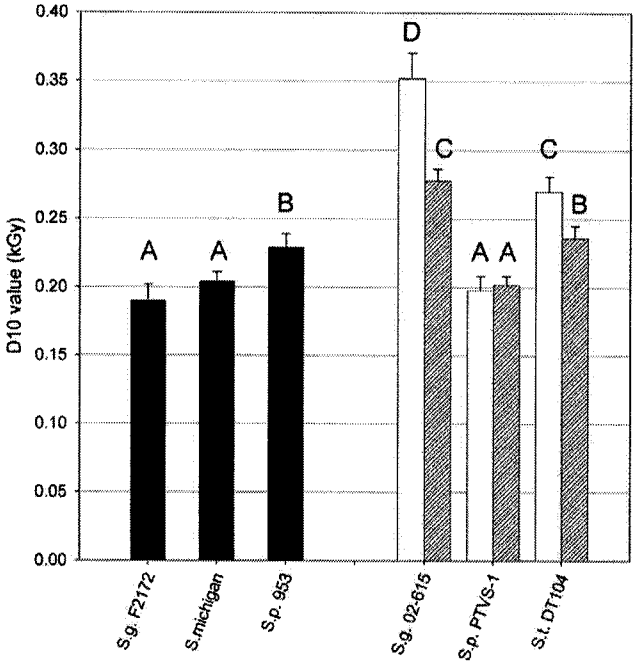

FIGURE 3. *Radiation D_{10}-values of* Salmonella *in phosphate buffer for Nal^R isolates (black), Nal^S isolates (white) and Nal^{Ri} isolates (gray). Error bars indicate regression SE. Bars with the same letter are not significantly different (ANCOVA, P > 0.05).*

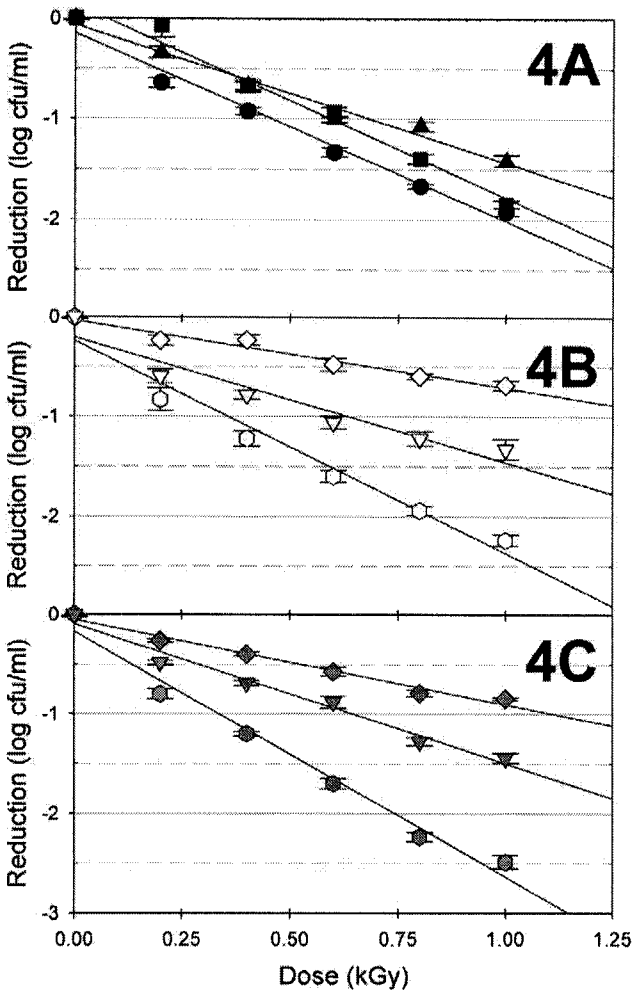

FIGURE 4. *Inactivation of* Salmonella *in orange juice by irradiation. (A) Nal^R isolates* Salmonella *Gaminara F2172 (black circle),* Salmonella *Michigan (black square), and* Salmonella *Poona 953 (black triangle). (B) Nal^S isolates* Salmonella *Gaminara 02-615 (white diamond),* Salmonella *Poona PTVS-1 (white hexagon), and* Salmonella *Typhimurium DT104 (white inverted triangle). (C) Nal^{Ri} isolates* Salmonella *Gaminara 02-615 (gray diamond),* Salmonella *Poona PTVS-1 (gray hexagon),* Salmonella *Typhimurium DT104 (gray inverted triangle). Bars indicate SE (n = 9 per data point).*

isolates, the Nal^R D_{10} (0.210 kGy) was significantly lower than that of the Nal^S (0.257 kGy). Induction of resistance to Nal caused a significant reduction in D_{10}-value for two of the three isolates (*Salmonella* Gaminara 0-615 and *Salmonella* Typhimurium DT104), with D_{10}-values ranging from 0.202 to 0.278 kGy (Fig. 3). The pooled Nal^{Ri} D_{10}-value (0.234 kGy) was significantly lower than that of the pooled D_{10} of the parent Nal^S strains and not significantly different from the pooled Nal^R D_{10}.

Irradiation also reduced the viable population of Nal^R (Fig. 4A), Nal^S (Fig. 4B), and Nal^{Ri} (Fig. 4C) isolates in orange juice. The reduction was dose-dependent. The resultant D_{10}-values were significantly different ($P < 0.05$) among the isolates. D_{10}-values were markedly higher for each isolate in orange juice than in buffer, with the ratio of juice D_{10}-value to buffer D_{10}-value ranging from 2.12 to 4.95, depending on the isolate. While the general pattern of response was somewhat similar to that obtained in buffer, the results were also more variable, with linear regression R^2 values ranging from 0.73 to 0.96. The variability of the data also resulted in wider ranges of statistical overlap and a wider range of variation between the high and low D_{10}-values seen for each group (Fig. 5). The Nal^R isolates had D_{10}-values ranging from 0.520 to 0.730 kGy, while the D_{10} of the Nal^S isolates ranged from 0.470 to 1.472 kGy (Fig. 5). When the data were pooled for each group of isolates, the Nal^R D_{10} (0.581 kGy) was significantly lower than that of the Nal^S (0.764 kGy). Induction of resistance to Nal caused a significant reduction in D_{10}-value for two of the three isolates (*Salmonella* Gaminara 02-615 and *Salmonella* Poona PTVS-1), with D_{10}-values ranging from 0.406 to 1.173 kGy (Fig. 5). The pooled Nal^{Ri} D_{10}-value (0.637 kGy) was intermediate in significance between that of the pooled D_{10} of the parent Nal^S strains, and the pooled Nal^R D_{10}, being not significantly different from either.

DISCUSSION

The use of antibiotic-resistant test strains coupled with selective culture media is a widely used technique for experimentation involving material with background microflora levels that may complicate recovery and enumeration. For the data obtained using these strains to be reflective of real-world conditions, the response of the derived resistant strain to the experimental circumstances must be similar to that of the parent sensitive strain. Whether created by transposon mutagenesis to insert an antibiotic resistance gene in a specific location of the genome, introduction of a resistance-gene–bearing plasmid, or by selection based on an

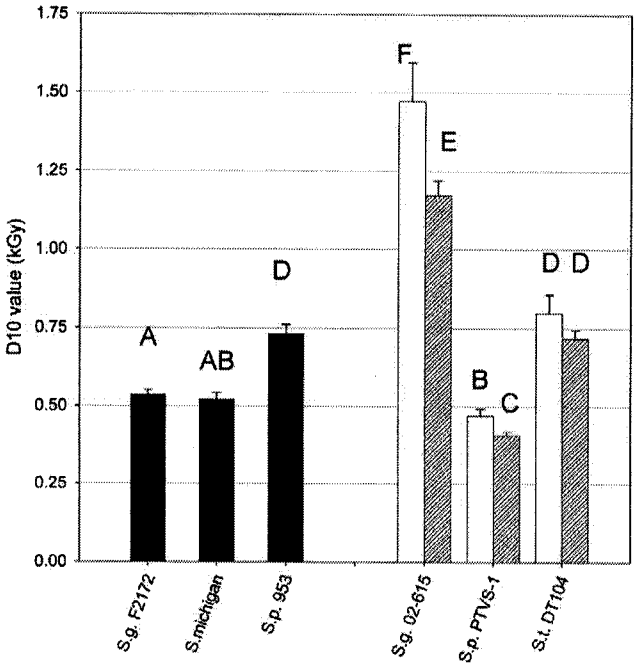

FIGURE 5. *Radiation* D_{10}*-values of* Salmonella *in orange juice for* Nal^R *isolates (black),* Nal^S *isolates (white), and* Nal^{Ri} *isolates (gray). Error bars indicate regression SE. Bars with the same letter are not significantly different (ANCOVA,* $P > 0.05$*).*

antibiotic resistant phenotype, these antibiotic-resistant strains are typically validated for use with only one particular type of antimicrobial intervention, such as chemical sanitizers *(3, 9, 19).* It should be recognized, however, that validation for a given antimicrobial intervention does not automatically translate into validation for another type of intervention. In published studies of inoculated and irradiated foods, resistance to Nal has been used as a selective marker with test strains of *E. coli* O157:H7 *(1, 4), Salmonella (1),* and *Listeria (2).* Validation of the application of Nal resistance within the context of irradiation is lacking.

It has recently been reported that induction of resistance to Nal among Nal^S isolates of *E. coli* O157:H7 results in significant reduction of the radiation D_{10}-value obtained when the pathogens were irradiated in neutral buffer and on green-leaf lettuce *(14).* In that study, the degree of reduction in D_{10}-value was specific to the *E. coli* O157:H7 isolate and the suspending test medium. In the present study, induction of resistance to Nal resulted in significantly reduced D_{10}-value for the Nal^S isolate *Salmonella* Gaminara 02-615 in both buffer and orange juice. For the Nal^S parent isolates *Salmonella* Typhimurium DT-104 and *Salmonella* Poona PTVS-1, derived Nal^{Ri} isolates were also observed to be more sensitive to irradiation than their respective Nal^S parents, but as the response was more pronounced in orange juice than in phosphate buffer, this response was inconsistent. This serves to highlight the complex and isolate-specific nature of the relationship between Nal resistance and response to irradiation, and difficulties of relating the behavior of the derived Nal^{Ri} isolates to that of their Nal^S parents.

Nalidixic acid and related quinolone antibacterial agents act by inhibiting DNA synthesis and replication by interfering with the action of DNA gyrase and topoisomerase IV *(5, 10).* The quinolone group is not known to significantly interfere with DNA repair. In contrast, the primary mode of action of ionizing radiation is via hydrogen and hydroxyl radical molecules resulting from the ionization of water molecules within the target—these radicals disrupt membranes, interfere with the functioning of proteins, and can lead to strand breakage of DNA *(15).* The effects of Nal are therefore seen as abnormal DNA synthesis and replication, rather than in DNA cleavage and subsequent repair as with radiation damage. Irradiation can result in double strand breaks in DNA and interstrand crosslinks, which are repaired by homologous recombination in *E. coli (12).* The RecBCD pathway is primarily responsible for *E. coli* conjugation and recombination, and requires a fully functional DNA gyrase to operate properly. Exposure to chemical stressors such as hydrogen peroxide and the DNA crosslinking agent mitomycin C increased the expression of the *gyrA* promoter fused to a *lacZ* gene *(16),* suggesting the possibility that radiation stress may lead to a similar induction which has a synergistic interaction with the mechanism that confers Nal resistance.

Although the alteration in D_{10}-values observed herein and with *E. coli* O157:H7 *(14)* suggest that the point of mechanistic commonality is related to the structure and function of nucleic acids, this remains a speculation until the molecular nature of the resistance is more fully elucidated. The precise mechanism(s) of the resistance to Nal in the Nal^{Ri} *Salmonella* isolates used herein has not been characterized on a molecular level. As the Nal^R mutants were generated by undirected selection, it is entirely likely that the specific form of resistance differs from isolate to isolate. This suggestion is supported by the slight but significant growth enhancement shown by *Salmonella* Typhimurium DT104-Nal^{Ri} at 10, 20, and 30 μg/ml Nal, relative to the 0-μg/ml control. A novel metabolic pathway which allows for the metabolism of Nal may be responsible for this slight increase in growth.

Resistance to Nal is a widely used marker in inoculation studies, and has been validated for a variety of antimicrobial treatments. The method of generating Nal^R mutants used in this study (i.e., successive culturing in increasing concentrations of Nal *(19)*), selects for the Nal^R phenotype. It is possible that this selection method may introduce other mutations, unrelated to the Nal^R phenotype, which are of significance in a condition of exposure to ionizing radiation, but not under exposure to chemical sanitizers or other interventions for which Nal^R has been validated. Thus, the increased sensitivity to ionizing radiation in Nal^R and Nal^{Ri} isolates may be a result of a more subtle change arising from the selection process. However, as with the possibility of an altered metabolism discussed above, confirmation of this speculation awaits further investigation of the molecular nature of the Nal^{Ri} isolates.

The range of D_{10}-values obtained in buffer and (with the exception of *Salmonella* Gaminara 02-615) in orange juice is generally consistent with values previously reported for *Salmonella (15).* D_{10}-values were higher in orange juice

than in phosphate buffer for all isolates. The increase in D_{10}-values was not related to natural or induced Nal resistance. The lowest ratio of juice D_{10}-value to buffer D_{10}-value (2.12) was seen with *Salmonella* Poona PTVS-1-Nal^{Ri}, while the highest ratio (4.95) was seen with *Salmonella* Gaminara 02-615-Nal^{Ri}. The variability of D_{10} among individual isolates and the complex influence of the suspending medium on the radiation D_{10}-value of associated bacteria are phenomena that have been reported before *(2, 13, 15)*. It has been previously observed that a medium with high antioxidant potential can quench the hydrogen and hydroxyl radicals produced during the irradiation process before they can act on suspended bacteria, thereby reducing the antimicrobial efficacy of irradiation *(11)*. However, due to the complex chemical nature of food products, a generalized predictive understanding of the effect of food chemistry on the radiation sensitivity of associated bacteria has yet to be formulated.

Resistance to one antibiotic may result in a certain level of cross protection against multiple different antibiotics. Golding and Matthews *(6)* showed that the vast majority of 52 strains of *E. coli* O157:H7, induced to be chloramphenicol resistant, were subsequently shown to have an increased tolerance to tetracycline, ciprofloxacin, and nalidixic acid. The potential significance of this cross-protection with regard to the efficacy of antimicrobial interventions (e.g., irradiation) is uncertain, particularly in the context of food substrates. As prevalence of antibiotic resistant pathogens increase *(6)*, information regarding the relative susceptibility of these resistant isolates to antimicrobial interventions becomes more important.

The use of Nal resistance as a marker was validated for use with chemical interventions and growth parameter measurements *(3, 19)*, but not for use with ionizing radiation. The significant reductions in D_{10}-values of the Nal^{Ri} versus the parent Nal^{S} strains observed in this study could lead to an overestimate of the efficacy of ionizing radiation against *Salmonella*. Radiation D_{10}-values are used as the basis for establishing recommended doses to achieve specific log reduction goals. Thus, a D_{10} which is significantly reduced will result in recommended doses that are inadequate to achieve the intended reductions in population. Using the D_{10}-values obtained for the Nal^{Ri} isolates in buffer, a radiation dose which is calibrated to achieve a 5-log reduction would instead result in reductions of the Nal^{S} parents of only 3.36 log for *Salmonella* Gaminara 02-615, 3.49 log for *Salmonella* Typhimurium DT-104, and 4.83 log for *Salmonella* Poona PTVS-1. A similar result is obtained when the recommended doses are based on the D_{10}-values obtained in orange juice. For *Salmonella* Gaminara 02-615, a 5-log dose of 5.86 kGy would not result in a 5-log reduction but only in a 3.97-log reduction. For *Salmonella* Typhimurium DT-104, the 5-log dose would result in only 4.48 log and for *Salmonella* Poona PTVS-1, only in a 4.32-log reduction. Thus, differences in D_{10}-values become magnified as they are used to calculate dose recommendations. This variation in efficacy can result in inadequate control of target microorganisms or negative sensory impact on treated foods.

The results presented herein support the conclusion made by Niemira *(14)* that the use of induced Nal resistance as a selective marker may be inappropriate for use in irradiation studies. However, the increased sensitivity of Nal^{R} and Nal^{Ri} *Salmonella* to ionizing radiation, relative to Nal^{S} parents, was more variable than that of Nal^{R} *E. coli* O157:H7 in the earlier study *(14)*. The significant differences in D_{10}-values resulting from induction of Nal resistance were as high as 25%, but were not significantly different in all cases. This raises the possibility that strain variation, uncharacterized mutations (unrelated to the Nal^{R} phenotype), and/or other factors may be influencing the radiation sensitivity of the isolates. Additional studies, using a larger set of *Salmonella* isolates, should be performed in the future to explore this phenomenon. Although a clear mechanism by which induction of resistance to Nal would alter the response of *Salmonella* to irradiation has not yet been developed, these results underscore the need for validation of derived "marker" strains against the particular antimicrobial intervention being studied.

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